

WHY BACTERIA MATTER: INSIGHTS FROM THE HYDRA HOLOBIONT

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SUMMARY

Animals, ranging from basal metazoans to primates, are engaged in symbiotic relationships with complex microbial ecosystems. These resident microbes influence fitness and thus ecologically important traits of their hosts, ultimately forming a meta-organism consisting of a multicellular host and a community of associated microorganisms. The evolutionary dynamics within such a meta-organism and the involved molecular interactions are rather complex and often difficult to investigate experimentally. Untangling the complex interactions requires simple animal models with only a few specific symbiotic partners. Here we show that organisms on at the base of the evolutionary scale such as the freshwater polyp Hydra may be key to dissecting the fundamental principles that underlie all host-microbe interactions.

FROM KARL AUGUST MÖBIUS TO THE HOLOGENOME THEORY OF EVOLUTION

In 1877, Karl Möbius, Professor of Zoology at Kiel University, coined the term “biocoenosis” for a community of living beings belonging to different species and associated by way of interspecies interdependence. In one of the first studies, later to become a classic, to be conducted in the emerging science of ecology, Möbius was seeking to determine why some oyster beds in the Atlantic were becoming exhausted, while the oyster beds in the British river estuaries and the Schleswig-Holstein oyster beds were very rich (*Möbius*, 1877). He related this phenomenon to the other species present, rather than to the oysters in the beds themselves. Möbius thus was the first to recognise that an ecological system must be taken as a whole and coined the term “biocoenosis” for a living community. About a hundred years

later it became obvious that not only ecological systems but also complex “environmental” diseases can only be understood if the relationships between the interacting infectious agents present at a given time in a given territory are recognized. By analogy with “biocoenosis”, the understanding of a disease as a complex dynamic phenomenon was conceptualized with the word “pathocoenosis” (*Grmek*, 1969).

Today we realize that all epithelia in animals are colonized by microbial communities and that, therefore, any multicellular organism must be considered a meta-organism comprised of the macroscopic host and synergistic interdependence with bacteria, archaea, fungi, and numerous other microbial and eukaryotic species. The «meta-organism» concept (*Bosch and McFall-Ngai*, 2011) considers the dynamic

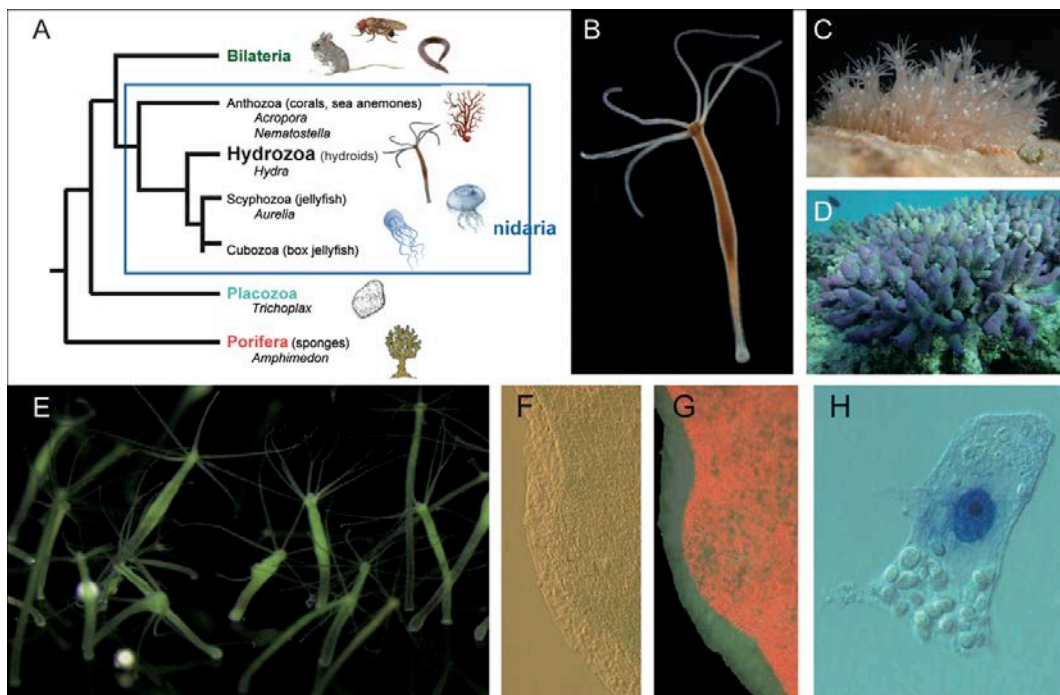


Figure 1: Cnidaria are a sister group of all Bilateria.

A: Phylogeny of basal metazoan animals.

B: *Hydra oligactis* (taken from Fraune and Bosch, 2007).

C: *Hydractinia milleri* (printed with permission from Gary McDonald).

D: Blue coral (taken from <http://www.jcu.edu.au/cgc/CoralGenomicsHP.html>).

E: *Hydra viridis* with *Chlorella* symbionts.

F: Phase-contrast micrograph of *Hydra viridis*.

G: Fluorescence microscopy of the same area shown in F. *Chlorella* algae appears red, *Hydra* tissue green.

H: Phase contrast micrograph of a single macerated endodermal epithelial cell containing symbiotic algae in the basal part below the nucleus (stained blue) (F-H taken from *Habetha et al 2003*).

communities of bacteria on epithelial surfaces as an integral part of the functionality of the respective organism itself. Today there is also an increasing appreciation that microbes are an essential part of the animal phenotype influencing fitness and thus ecologically-important traits of their hosts (*O'Hara and Shanahan, 2006; McFall-Ngai, 2007; Fraune and Bosch, 2010*). Disease onset is seen as a complex set of interactions among a variety of associated partners that affect the fitness of the collective holobiont (*Rosenstiel et al., 2009*). Discovering that individuals

are not solitary, homogenous entities but consist of complex communities of many species that likely evolved during a billion years of coexistence led to the hologenome theory of evolution (*Rosenberg et al., 2007, 2009; Zilber-Rosenberg and Rosenberg, 2008*) which considers the holobiont with its hologenome as the unit of selection in evolution. Thus, modern symbiosis research has become an emerging cross-disciplinary field focused on understanding the general principles by which these complex host-microbe communities function and evolve.

What is the complexity in species number and structural organization of these associations? What is the physiological role of temporal differences of associated microbiota during life cycles? Which selective forces drive the evolution of these interactions, i.e. how do the associated organisms influence each other's fitness? Which forces shape the colonizing microbial compo-

sition? Finally, what are the consequences of the associations on molecular pathways and the reactive genomes? Here we show that for addressing these questions and untangling the complex interactions that influence the host's health and development, members of the ancient animal phylum Cnidaria may serve as simple but highly informative models.

THE HYDRA HOLOBIONT

Hydra is member of the animal phylum Cnidaria which are not only among the earliest known phyletic lineages known to contain stem cells (Figure 1A) but also possess most of the gene families found in bilaterians and have retained many ancestral genes that have been lost in *Drosophila* and *C. elegans* (Kortschak et al., 2003; Miller et al., 2005; Technau et al., 2005; Putnam et al., 2007; Hemmrich et al., 2012). Similar to other animals, Cnidaria are complex holobionts consisting of the animal and its associated endogenous microbiota. Inter-species interactions in several Cnidaria species (Figure 1A-1D) between symbiotic algae and host cells have been the subject of research since decades since they not only provide insights into the basic "tool kit" necessary to establish symbiotic interactions, but are also of relevance in understanding the resulting evolutionary selection processes (e.g. Muscatine and Lenhoff, 1963; Pool, 1979; Thorington and Margulis, 1981; O'Brien, 1982; for review see: Bosch, 2012a). In the meantime it is becoming evident that in Cnidaria such as green *Hydra viridis* (Figure 1E-1H) or many coral species, a long term persistence of mutualistic associations is prevalent not only in

two-party interactions of polyp and symbiotic algae, but also in more complex systems comprising three or more associates including bacteria and viruses (Bosch, 2012a, 2012b). Thus, beside photosynthetic algae (Figure 1G-1H), bacteria are another important component of the cnidarian holobiont. In Hydra, the 36 identified bacterial phylotypes represent three different bacterial divisions and are dominated by Proteobacteria and Bacteroidetes (Fraune and Bosch, 2007, 2010). Disturbance or shifts in any of these partners can compromise the health of the whole animal (Fraune et al., 2009). Loss of symbiotic algae from coral tissues, for example, can lead to coral bleaching and death. Since healthy individuals of the same coral species from different location are colonized by similar bacterial communities (Rohwer et al., 2002) but diseased or bleached corals contain changed bacterial communities that differ greatly from healthy ones (Ritchie, 2006; Rosenberg et al., 2007), it seems that similar to complex "environmental" diseases in human, understanding diseases within corals requires an in-depth knowledge of the basic biology of each holobiont member.

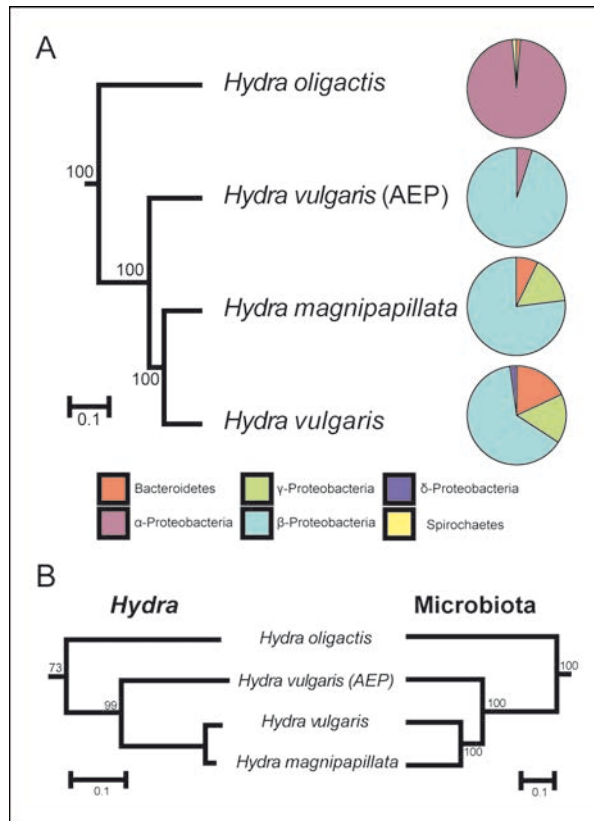


Figure 2: Hydra polyps are colonized by species-specific microbiota. A: Bacterial communities identified from four different Hydra species. B: Comparison of the phylogenetic tree from Hydra and the environmental cluster tree of the corresponding microbiota.

THE HOST ACTIVELY SHAPES THE COLONIZING MICROBIOTA

For decades a number of Hydra species have been cultivated under standard conditions at constant temperature and identical food. It came as a complete surprise, therefore, that examining the microbiota in different Hydra species kept in the laboratory for more than 20 years under controlled conditions revealed an epithelium colonized by a complex community of microbes, and that individuals from different species differed greatly in their microbiota. Even more astonishing was the finding that individuals living in the wild were colonized by a group of microbes that is similar to that in polyps grown in the lab, pointing to the maintenance of spe-

cific microbial communities over long periods of time. Bacteria in Hydra are specific for any given species (Figure 2A) (Fraune and Bosch, 2007; Fraune et al., 2010). Closely related Hydra species as *Hydra vulgaris* and *Hydra magnipapillata* are associated with a very similar microbial community. In contrast, *Hydra oligactis*, the most basal Hydra species analysed so far (Hemrich et al., 2007), is associated with the most distinct microbial community compared to the other Hydra species. In line with this, comparing the phylogenetic tree of the Hydra species with the according cluster tree of associated bacterial communities reveals a

very similar microbial community. In contrast, *Hydra oligactis*, the most basal Hydra species analysed so far (Hemrich et al., 2007), is associated with the most distinct microbial community compared to the other Hydra species. In line with this, comparing the phylogenetic tree of the Hydra species with the according cluster tree of asso-

ciated bacterial communities reveals a high degree of congruency (Figure 2B). This strongly indicates that distinct selective pressures are imposed on and within the Hydra epithelium. The forces that shape the colonizing microbial composition are the focus of much current investigation (Bevins and Salzman, 2011).

HOW DOES THE HOST CONTROL THE MICROBIOTA IN THE CONTEXT OF SPECIFIC DEVELOPMENTAL OR ENVIRONMENTAL CONDITIONS?

In the same way that microbial communities are expected to change in different parts of a body, they are also dynamic in time. For a first understanding of the temporal dynamics in Hydra-microbe interactions we investigated the establishment of the microbiota during oogenesis and embryogenesis. Early embryonic stages in Hydra are colonized by a limited number of microbes (Fraune et al., 2010). During embryogenesis the number of bacterial colonizers changes in number and composition. For example, *Curvibacter*-related re present only in late developmental stages while they appear to be absent in the early embryo. Thus, early developmental stages have a microbiota that is clearly distinct from later developmental stages. Interestingly, the differential colonization is reflected in differences in antimicrobial activity. Hydra embryos are protected by a maternally produced antimicrobial peptide (AMP) of the periculin peptide family, which controls the establishment of the microbiota during embryogenesis. Beginning with the gastrula stage, Hydra embryos express a set of periculin peptides (periculin 2a and 2b), which replaces the maternal produced periculin peptides 1a and 1b. This shift in the expression within the periculin peptide

family represents a shift from maternal to zygotic protection of the embryo (Fraune et al., 2011). In adult Hydra polyps, additional AMPs including hydramacin (Bosch et al., 2009) and arminin (Augustin et al., 2009) contribute to the host-derived control of bacterial colonization.

After hatching from the “cutical” stage the Hydra polyps get colonized by its specific bacterial community. The processes controlling community membership and influencing the establishment of the microbial ecosystem during development are poorly understood. Therefore, the microbial communities in polyps at various time points after hatching was profiled (Franzenburg et al., 2013). Distinct features included high diversity of community profiles in the first week, followed by progressive emergence of a stable adult-like pattern characterized by low species diversity and the preponderance of the Betaproteobacterium *Curvibacter*.

In adult Hydra polyps, additional AMPs including hydramacin (Bosch et al., 2009) and arminin (Augustin et al., 2009) and the bacterial signalling via MyD88 (Franzenburg et al., 2012) contribute to the host-derived control of bacterial colonization.

ANTIMICROBIAL PEPTIDES - KEY FACTORS FOR HOST-BACTERIA CO-EVOLUTION

Antimicrobial peptides (AMPs) are known as prominent effector molecules which get often secreted after external stimuli. Do they have, in addition to their killing activity against pathogens, key regulatory functions in host-microbe homeostasis as the driving force that leads to changes in microbiota composition? To investigate whether the ectopic expression of an AMP may affect the number and composition of the colonizing microbiota at the ectodermal epithelial surface, we generated transgenic Hydra expressing periculin1a in ectoderm epithelial cells (*Fraune et al., 2010*). Comparing the bacterial load of these transgenic polyps with that of wild-type control polyps revealed not only a significantly lower bacterial load in transgenic polyps overexpressing periculin1a but also, unexpectedly, drastic changes in the bacterial community structure. Analysing the identity of the colonizing bacteria showed that the dominant β -Proteobacteria decreased in number, whereas α -Proteobacteria were more prevalent. Thus, overexpression of periculin causes not only a decrease in the number of associated bacteria but also a changed bacterial composition. With the transgenic polyps overex-

pressing periculin we apparently have created a new holobiont that is different from all investigated Hydra species. From these results we assume that specific associations between hosts and bacteria are a result of bacterial adaptation to different repertoires on AMPs in different host species. Evolutionary changes in the AMP repertoire of host species, therefore, are expected to lead to changes in the composition of the associated bacterial community. Future efforts will be directed towards analysing the performance of this new phenotype under different environmental conditions. Interestingly, patients with Crohn's disease often have strongly reduced α -defensin expression and drastically altered endogenous microbiota (*Wehkamp et al., 2005*). Moreover, mice expressing human alpha-defensin-5 (DEFA5) and mice lacking an enzyme required for the processing of mouse alpha-defensins show significant changes in intestinal microbiota composition (*Salzman et al., 2010*). These findings support the view that epithelial-derived AMP may represent an important regulatory mechanism shaping the composition of epithelial microbiota.

WHAT ARE THE MICROBES FOR?

The intimacy of the interaction between host and microbiota, as well as the high evolutionary pressure to maintain a specific microbiota, points to the significance of the interkingdom association and implies that hosts deprived of their microbiota should be at a disadvantage. To investigate the effect of absence of microbiota in Hydra we have produced gnotobiotic Hydra

polyps that are devoid of any bacteria. While morphologically no differences could be observed to control polyps, we are currently finding evidence that Hydra lacking bacteria suffer from fungal infections unknown in normally cultured polyps (*Franzenburg, Fraune and Bosch, unpublished*). Thus, do beneficial microbes associated with Hydra produce anti-fungal compounds?

Future efforts are directed towards isolating the active substances from these bacteria that eventually may lead to the development of novel antimycotics.

Microbes also provide signals for multiple developmental steps. One of the most pervasive examples of microbial impact in animal development is in the induction of settlement and metamorphosis of many marine invertebrate larvae (Hadfield, 2011). This transition is an absolute requirement for completion of the animal's life cycle, and is dependent upon induction by exogenous morphogenetic cues, many of which are produced by bacteria associated with a particular environmental surface. Hydractinia, for example, (Figure 1C) a marine colonial Cnidaria frequently found in the North Sea, commonly covers shells inhabited by hermit crabs. Fertile colonies, male and female, produce eggs and sperm, respectively, and within less than three days the fertilized egg develops into a mature planula larva. "Mature" larva means a larva that is able to metamorphose into a polyp, but under sterile laboratory conditions it will never do. It will rather die, as it is unable to take up food (Leitz and Wagner, 1993; Walther et al., 1996; Frank et al., 2001). To

continue its development, it needs an external trigger that appears to be provided in the natural habitat by certain sedentary bacteria of the genus *Alteromonas*. A lipophilic substance produced by these bacteria is thought to act as this trigger (Leitz and Wagner, 1993). The mechanisms by which Hydractinia sense bacteria-derived environmental cues to form colonies and to reproduce may provide crucial insights into the genetic and developmental foundations of life cycles, but little is known about their natural history or biochemistry. Observations in a number of other invertebrates and vertebrates strongly support the view that microbes should be considered partners in animal development. Bacterial contributions are indispensable, for example, in shaping the immune system and development of organs such as the vertebrate intestine or the squid light organ (reviewed in Fraune and Bosch, 2010). Animal development has traditionally been viewed as an autonomous process directed by the genome. It seems that we have to rethink development at least in part, as an orchestration of both animal-encoded ontogeny and inter-kingdom communication.

THE HOLOBIONT IN A CONSTANTLY CHANGING ENVIRONMENT

The association between host and microbes is strongly affected by the environment. To determine the impact of different environmental conditions on the bacterial community in Hydra, we cultured polyps, which were taken from the wild, for two months under standard laboratory conditions. Thereafter, we analysed the associated bacteria in comparison to the bacteria from polyps taken directly from the wild. Culturing of polyps from the wild under laboratory conditions involves a change in culture temperature, culture medium

and food source. These changes have significant effects on the composition of the bacterial community. For example, while one bacterial phylotype belonging to the α -Proteobacteria could be identified as the most dominant species in long term culture, in polyps from the wild and two month after the shift to the laboratory this bacterium was present only in relative low abundance (Fraune and Bosch, 2007). Other bacterial species completely disappeared from the tissue due to the change in culturing conditions. Thus,

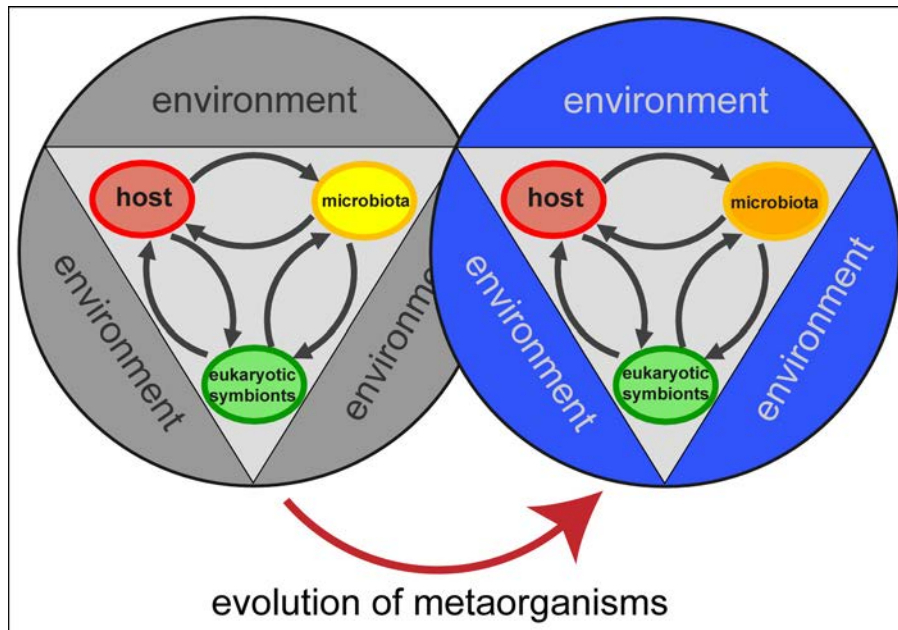


Figure 3: The meta-organism under changing environmental conditions

Hydra is not only associated with species specific bacteria but also responds to changes in the environment with changes in the bacterial community. In sum, the holobiont appears to be a dynamic system being characterized by functional redundancy and fast adaptations to altered environmental conditions.

Based on the holobiont concept, Rosenberg and colleagues in 2007 proposed that corals are able to adapt rapidly to changing environmental conditions by altering their associated microbiota (Figure 3) (Rosenberg et al., 2007). Depending on the variety of different niches provided by the host, which can change with developmental stage, diet or other environmental factors, a more or less diverse microbial community can be established within a

given host species. Since this, for example, may provide corals with resistance against certain pathogens enabling them to adapt much faster to novel environmental conditions than by mutation and selection, host-microbe interactions may be considered as significant drivers of animal evolution and diversification. This hypothesis is supported by at least three observations: (i) corals are associated with diverse microbiota (Rohwer et al., 2002; Bourne et al., 2008); (ii) the associated microbiota change in response to environmental change in response to environmental stress (Ritchie and Smith, 1995; Pantos et al., 2003) or seasons (Koren and Rosenberg, 2006); and (iii) corals are able to develop resistance against pathogens although they lack adaptive immune response (Reshef et al., 2006).

CONCLUDING REMARKS

The beneficial microbiota is a complex and multifunction ecosystem that is es-

sential to the development, protection, and overall health of its host. Thus, the

microbiota appears to function as an extra organ, to which the host has outsourced numerous crucial metabolic, nutritional, and protective functions. Studies from Cnidaria to primates indicate that the host's role far outweighs other environmental factors in molding the composition of the microbiota. Antimicrobial peptides appear to be key factors for host-bacteria co-evolution and the driving force that leads to changes in microbiota composition. Finally, and maybe most important, the dynamic relationship between symbiotic microorganisms and environmental conditions results in the selection of the most advantageous holobiont. In corals, changing their microbial partners may allow them to adapt to changing environmental conditions much more rapidly than via mutation and selection (Figure 3).

Taken together, studying host-microbe interactions in basal metazoans is a challenging and exciting field of sym-

biosis research. Cnidaria not only offer valuable models for exploring the basis of interkingdom-communication and the role of bacterial signalling in animal development. Findings derived from the *in vivo* context of the Cnidaria models may also provide one of the simplest possible systems to address questions of how a stable host-microbe community is established and remains in balance over time. The uncovered basic molecular machinery can be transliterated to more complex organisms, providing conceptual insights into the complexity of host-microbe interactions. Symbiosis research in Cnidaria, therefore, is an emerging field in which scientists from many disciplines can make fundamental discoveries and rapidly advance scientific understanding of a strictly microbe-dependent life style and its evolutionary consequences while combining laboratory and field studies.

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LITERATURE

- Augustin, R., Anton-Erxleben, F., Jungnickel, S., Hemmrich, G., Spudy, B., Podschun, R., and Bosch, T.C.: Activity of the novel peptide arminin against multiresistant human pathogens shows the considerable potential of phylogenetically ancient organisms as drug sources. *Antimicrob. Agents Chemother.* 53, 5245-5250 (2009).
- Bevins, C.L. and Salzman, N.H.: The potter's wheel: the host's role in sculpting its microbiota. *Cell. Mol. Life Sci.* 68, 3675-3685 (2011).
- Bosch, T.C.G.: What Hydra has to say about the role and origin of symbiotic interactions. *Biol. Bull.* 223, 78-84 (2012a)
- Bosch, T.C.G.: Understanding complex host-microbe interactions in Hydra. *Gut Microbes* 3, 345-351 (2012b).
- Bosch, T.C., Augustin, R., Anton-Erxleben, F., Fraune, S., Hemmrich, G., Zill, H., Rosenstiel, P., Jacobs, G., Schreiber, S., Leippe, M., Stanisak, M., Grötzinger, J., Jung, S., Podschun, R., Bartels, J., Harder, J., and Schröder, J.M.: Uncovering the evolution-

- ary history of innate immunity: the simple metazoan *Hydra* uses epithelial cells for host defence. *Dev. Comp. Immunol.* 33, 559-569 (2009).
- Bosch, T.C. and McFall-Ngai, M.J.: Metaorganisms as the new frontier. *Zoology (Jena)* 114, 185-190 (2011).
- Bourne, D., Iida, Y., Uthicke, S., and Smith-Keune, C.: Changes in coral-associated microbial communities during a bleaching event. *ISME J.* 2, 350-363 (2008).
- Frank, U., Leitz, T., and Muller, W.A.: The hydroid *Hydractinia*: a versatile, informative cnidarian representative. *Bioessays* 23, 963-971 (2001).
- Franzenburg, S., Fraune, S., Altrock, P.M., Kunzel, S., Baines, J.F., Traulsen, A., and Bosch, T.C.: Bacterial colonization of *Hydra* hatchlings follows a robust temporal pattern. *ISME J.* 7, 781-790 (2013).
- Fraune, S., Abe, Y., and Bosch, T.C.: Disturbing epithelial homeostasis in the metazoan *Hydra* leads to drastic changes in associated microbiota. *Environ Microbiol* 11, 2361-2369 (2009).
- Fraune, S., Augustin, R., Anton-Erxleben, F., Wittlieb, J., Gelhaus, C., Klimovich, V.B., Samoilovich, M.P., and Bosch, T.C.: In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. *Proc. Natl. Acad. Sci. USA* 107, 18067-18072 (2010).
- Fraune, S., Augustin, R., and Bosch, T.C.: Embryo protection in contemporary immunology: Why bacteria matter. *Commun. Integr. Biol.* 4, 369-372 (2011).
- Fraune, S. and Bosch, T.C.: Long-term maintenance of species-specific bacterial microbiota in the basal metazoan *Hydra*. *Proc. Natl. Acad. Sci. USA* 104, 13146-13151 (2007).
- Fraune, S. and Bosch, T.C.: Why bacteria matter in animal development and evolution. *Bioessays* 32, 571-580 (2010).
- Grmek, M.D.: Préliminaire d'une étude historique des maladies. *Annales ESC* 24, 1437-1483 (1969).
- Hadfield, M.G.: Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Ann. Rev. Mar. Sci.* 3, 453-470 (2011).
- Hemmerich, G., Anokhin, B., Zacharias, H., and Bosch, T.C.: Molecular phylogenetics in *Hydra*, a classical model in evolutionary developmental biology. *Mol. Phylogenet. Evol.* 44, 281-290 (2007).
- Hemmerich, G., Khalturin, K., Boehm, A.M., Puchert, M., Anton-Erxleben, F., Wittlieb, J., Klostermeier, U.C., Rosenstiel, P., Oberg, H.H., Domazet-Lošo, T., Sugimoto, T., Niwa, H., and Bosch, T.C.G.: Molecular signatures of the three stem cell lineages in *Hydra* and the emergence of stem cell function at the base of multicellularity. *Mol. Biol. Evol.* 29, 3267-3280 (2012).
- Koren, O. and Rosenberg, E.: Bacteria associated with mucus and tissues of the coral *Oculina patagonica* in summer and winter. *Appl. Environ. Microbiol.* 72, 5254-5259 (2006).
- Kortschak, R.D., Samuel, G., Saint, R., and Miller, D.J.: EST analysis of the cnidarian *Acropora millepora* reveals extensive gene loss and rapid sequence divergence in the model invertebrates. *Curr Biol.* 13, 2190-2195 (2003).
- Leitz, T. and Wagner, T.: The marine bacterium *Alteromonas espejiana* induces metamorphosis of the hydroid *Hydractinia echinata*. *Mar. Biol.* 115, 173-178 (1993).
- McFall-Ngai, M.: Adaptive immunity: care for the community. *Nature* 445, 153 (2007).
- Miller, D.J., Ball, E.E., and Technau, U.: Cnidarians and ancestral genetic complexity in the animal kingdom. *Trends Genet.* 21, 536-539 (2005).
- Möbius, K.A.: Die Auster und die Austernwirtschaft. Wiegandt, Hempel and Parey, Berlin, Germany (1877).
- Muscatine, L. and Lenhoff, H.M.: Symbiosis: On the role of algae symbiotic with *Hydra*. *Science* 142, 956-958 (1963).
- O'Brien, T.L.: Inhibition of vacuolar membrane fusion by intracellular symbiotic algae in *Hydra viridis* (Florida strain). *J. Exp. Zool.* 223, 211-218 (1982).
- O'Hara, A.M. and Shanahan, F.: The gut flora

- as a forgotten organ. *EMBO Rep.* 7, 688-693 (2006).
- Pantos, O., Cooney, R.P., Le Tissier, M.D., Barer, M.R., O'Donnell, A.G., and Bythell, J.C.: The bacterial ecology of a plague-like disease affecting the Caribbean coral *Montastrea annularis*. *Environ. Microbiol.* 5, 370-382 (2003).
- Pool, R.R.: The role of algal antigenic determinants in the recognition of potential algal symbionts by cells of chlorohydra. *J. Cell. Sci.* 35, 367-379 (1979).
- Putnam, N.H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V.V., Jurka, J., Genikhovich, G., Grigoriev, I.V., Lucas, S.M., Steele, R.E., Finnerty, J.R., Technau, U., Martin-dale, M.Q., AND Rokhsar, D.S.: Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* 317, 86-94 (2007).
- Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I., and Rosenberg, E.: The coral probiotic hypothesis. *Environ. Microbiol.* 8, 2068-2073 (2006).
- Ritchie, K.B.: Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar. Ecol. Prog. Ser.* 322, 1-14 (2006).
- Ritchie, K.B. and Smith, W.G.: Carbon-source utilization of coral-associated marine heterotrophs. *J. Mar. Biotechnol.* 3, 107-109 (1995).
- Rohwer, F., Seguritan, V., Azam, F., and Knowlton, N.: Diversity and distribution of coral-associated bacteria. *Mar. Ecol. Prog. Ser.* 243, 1-10 (2002).
- Rosenberg, E., Koren, O., Reshef, L., Efrony, R., and Zilber-Rosenberg, I.: The role of microorganisms in coral health, disease and evolution. *Nat. Rev. Microbiol.* 5, 355-362 (2007).
- Rosenberg, E., Sharon, G., and Zilber-Rosenberg, I.: The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. *Environ. Microbiol.* 11, 2959-2962 (2009).
- Rosenstiel, P., Philipp, E.E., Schreiber, S., and Bosch, T.C.: Evolution and function of innate immune receptors--insights from marine invertebrates. *J. Innate Immun.* 1, 291-300 (2009).
- Salzman, N.H., Hung, K., Haribhai, D., Chu, H., Karlsson-Sjoberg, J., Amir, E., Teg-gatz, P., Barman, M., Hayward, M., Eastwood, D., Stoel, M., Zhou, Y., Sodergren, E., Weinstock, G.M., Bevins, C.L., Williams, C.B., and Bos, N.A.: Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* 11, 76-83 (2010).
- Technau, U., Rudd, S., Maxwell, P., Gordon, P.M., Saina, M., Grasso, L.C., Hayward, D.C., Sensen, C.W., Saint, R., Holstein, T.W., Ball, .EE., and Miller, D.J.: Maintenance of ancestral complexity and non-metazoan genes in two basal cnidarians. *Trends Genet.* 21, 633-639 (2005).
- Thorington, G. and Margulis, L.: *Hydra viridis*: transfer of metabolites between Hydra and symbiotic algae. *Biol. Bull.* 160, 175-188 (1981).
- Walther, M., Ulrich, R., Kroiher, M., and Berking, S.: Metamorphosis and pattern formation in *Hydractinia echinata*, a colonial hydroid. *Int. J. Dev. Biol.* 40, 313-322 (1996).
- Wehkamp, J., Salzman, N.H., Porter, E., Nuding, S., Weichenthal, M., Petras, R.E., Shen, B., Schaeffeler, E., Schwab, M., Linzmeier, R., Feathers, R.W., Chu, H., Lima, H. Jr., Fellermann, K., Ganz, T., Stange, E.F., and Bevins, C.L.: Reduced Paneth cell alpha-defensins in ileal Crohn's disease. *Proc. Natl. Acad. Sci. USA* 102, 18129-18134 (2005).
- Zilber-Rosenberg, I. and Rosenberg, E.: Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* 32, 723-735 (2008).